the cytoplasmic side was observed. However, an atomic model of the outward-facing (OF) state of GlpT and the IF-to-OF transition pathway remain unknown. Here we have used a novel approach based on nonequilibrium MD simulations to drive the transition of GlpT from its available IF state to its unknown OF state in an explicit membrane environment. Based on the interhelical symmetries, a set of non-conventional collective variables were defined for the transmembrane helices to specifically reflect the conformational changes involved during the IF-to-OF transition. The system was then steered along these collective variables in a nonequilibrium scheme. The obtained IF-to-OF transition pathway was further optimized adaptively by minimizing the nonequilibrium work, which is a good way to produce more realistic transition pathways. Thus, the protocol of inducing the transition was optimized after each simulation based on the resulted trajectory. Nonequilibrium work relations were used to analyze the trajectories and extract information about the OF state structure, the IF-to-OF transition mechanism, the intermediate/occluded states, and the characteristics of the OF-state binding site, providing insight about the general mechanism of MFS transporters and more generally introducing a novel approach to study the IF-to-OF transitions in membrane transporters.

3080-Plat
Mechanism of the Alternating Access in Leut-Fold Na^+-Coupled Secondary Transporters: A Computational Transition Path Study
Chunfeng Zhao, Sergei Yu Noskov.
University of Calgary, Calgary, AB, Canada.
Na+-coupled LeuT-fold secondary transporters consist of many essential membrane proteins that utilize the concentration gradient of Na+ to transport solutes across the cell membrane against their concentration gradients. The solutes they transport include many physiologically important molecules such as neurotransmitters (serotonin, dopamine, etc.), sugar molecules, nucleotide bases, etc. Dysfunction of these transporters is implicated in various diseases and syndromes and they are targets of many clinical drugs including antidepressants. Recent progress in crystallographic studies of the LeuT structural family has revealed striking similarities in the structural organization of ion and solute binding, implying some general mechanisms of substrate translocation and transporter conformational changes. This presentation will focus on the transport mechanism of transporters in this structural family, featuring Mhp1, a bacterial homologue of cation nucleotide-base symporters. Three crystal structures of this protein in open-to-out, occluded-out, and open-to-in conformational states have become available recently [1, 2]. The optimal transition path connecting these states is obtained from the ''string method with swarm of trajectories'' [3]. Potential of Mean Force (PMFs) maps are computed to characterize the path. Free energy simulations reveal the coupling between the co-transported ion and the main substrate and a profile of the dynamical interactions between substrates, ions, and the protein. This computational study will reveal the molecular mechanisms that govern the transporter's conformational changes from open to one face of the membrane to open to the opposite face.
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3081-Plat
Structure, Dynamics, and Mechanism of the Leucine Transporter Studied by Double Electron Electron Resonance Spectroscopy
Kelli Kazmier[1], Matthias Quick[2], Lei Shi[3], Harel Weinstein[3], Jonathan A. Javitch[2], Hassane S. Mchaourab[1].
[1]Vanderbilt University, Nashville, TN, USA, [2]Columbia University College of Physicians and Surgeons, New York, NY, USA, [3]Weill Medical College of Cornell University, New York, NY, USA.
The neurotransmitter/sodium symporter (NSS) family of transporters includes the human dopamine, serotonin, and norepinephrine transporters. These transporters harness sodium and chloride gradients to facilitate reuptake of neurotransmitters from the synapse thus terminating neurotransmission. Due to their critical regulatory role, NSSs are the targets of numerous psychiatric therapeutics and drugs of abuse. Progress in understanding and treating diverse conditions including depression and anxiety will require a detailed description of the structure and function of this class of proteins. Structural investigations of NSS have focused on a bacterial homolog of these transporters, LeuT. This work will describe a systematic investigation of the intracellular dynamics of LeuT as well as detail progress in defining the LeuT transport mechanism and open-inward structure. Using double electron-electron resonance (DEER) spectroscopy, a pulsed EPR technique, over 40 double mutants were analyzed for their distance distributions and relative dynamics. Mechanistic descriptions were inferred by tracking shifts in equilibria between multi-component distance distributions and relating these shifts to biochemical conditions across all mutants. Closed-inward distances were largely consistent with the LeuT crystal structures, while primary gating motions were identified at helices 1 and 6 with additional reorientations of helices 5 and 7, resulting in the open-inward structure. Distance data are being implemented into restrained computational modeling to generate preliminary models of the open-inward conformation of LeuT. These results will be compared to the current models of LeuT open-in structure and transport mechanism derived using alternative approaches.

3082-Plat
Conformational Dynamics in the Transport Cycle of Leucine Transporter on the Extracellular-Side
Shruti Sharma[1], Richard A. Stein[1], Matthias Quick[2], Sebastian Stolzenberg[3], Lei Shi[3], Harel Weinstein[3], Jonathan A. Javitch[2], Hassane S. Mchaourab[1].
[1]Vanderbilt University, Nashville, TN, USA, [2]Center for Molecular Recognition, Columbia University, New York, NY, USA, [3]Weill Cornell Medical College, New York, NY, USA.
LeuT is a bacterial homolog of the Neurotransporter:Sodium Symporters (NSS) which mediates the sodium dependent re-uptake of neurotransmitters from the synaptic cleft thereby terminating neuronal transmission. LeuT has been crystallized bound with various amino acids (leucine, glycine, methionine, tyrosine and tryptophan), tricyclic antidepressants (TCA), and selective serotonin reuptake inhibitors (SSRIs). Although these structures reveal molecular details of ion/substrate binding and inhibition, the conformational dynamics underlying a complete transport cycle have yet to be elucidated. Computational modeling studies have led to a proposal of a rocking bundle mechanism that is based on the inherent inverted pseudo-symmetry between TM1-5 and TM6-10. According to this model, TM1, 2, 6, and 7 form a bundle that tilts with respect to other surrounding helices and results in closing and opening of cytoplasmic and extracellular molecular gates. We are investigating the conformational changes of transmembrane helices predicted to move at different stages of the transport cycle by measuring distances between spin labels. Monitoring these dynamics should provide insights into molecular interactions that govern the extracellular gating of the transporter during the transport process. Our results show that the bundle does not behave as a single rigid body structural unit as previously proposed. Some TM helices show a distinct increase in conformational flexibility as observed from the width of distance distributions, whereas others show a change in the equilibrium between different conformational intermediates in response to ion and or substrate binding. We are also analyzing these conformational changes with respect to the crystal structure through geometric optimization in order to consolidate distance changes into a model of the sodium bound intermediate.

3083-Plat
Mapping Conformational Changes Associated with the Catalytic Cycle of Human P-Glycoprotein (ABCB1)
Jaya Bhatnagar[1], Hong-May Sim[1], Elka Georgieva[2], Khyati Kapoor[1], Eduardo Chufan[1], Shinobu Ohnuma[1], Peter P. Borbat[2], Jack H. Freed[2], Zuben E. Sauna[3], **Suresh V. Ambudkar**[1].
[1]National Cancer Institute, NIH, Bethesda, MD, USA, [2]National Biomedical Center for Advanced ESR Technology, ACERT, Cornell University, Ithaca, NY, USA, [3]Division of Hematology, CBER, US Food and Drug Administration, Bethesda, MD, USA.
P-glycoprotein (Pgp, ABCB1) is an ABC transporter that uses the energy of ATP hydrolysis to efflux a variety of amphipathic substrates including anti-cancer agents from the cell. In this study, we have used disulfide crosslinking and Pulsed Dipolar Electron Spin Resonance Spectroscopy (PDS) to elucidate the conformational changes associated with the catalytic cycle. We generated a library of double cysteine mutants in a cys-less background and expressed in baculovirus-insect and mammalian cell expression systems. All the mutants expressed at the cell surface in HeLa cells and were also functionally active. Disulfide crosslinking was performed in crude membranes and each double cysteine mutant of Pgp was tested for its ability to crosslink with ten crosslinkers of varied spacer arm length. We observed crosslinking in four double cysteine mutants with Cys residues in TM3/TM9 (T173C/T816C), ICL2/NBD2